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A novel molecularly imprinted polymer thin film as biosensor for uric acid

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ABSTRACT

A novel amine–imide type conducting polymer, denoted as poly(PD–BCD), was molecularly imprinted on an indium–tin oxide (ITO) glass, with uric acid (UA) as the template and without any functional monomer. Intending to improve the imprinting efficiency, the polymer content was varied from 0.3 to 0.9 wt% during the preparation of the molecularly imprinted polymer (MIP), thereby varying the thickness of the polymer film; the content of UA as the template was maintained to be the same for all the films. The sensitivities of the thus prepared MIP electrodes were calculated to be more than 3-fold, compared to those of the corresponding non-MIP (NMIP) electrodes, which were obtained through the same method, however, without adding UA during their preparation. A polymer content of 0.6 wt% rendered the best performing MIP electrode, as judged by the imprinting efficiency and sensitivity of the electrode for UA. A linear relationship between steady-state currents and UA concentrations from 0 to 1.125 mM was obtained for both types of the sensors. The sensitivities of the MIP and the NMIP electrodes made with 0.6 wt% of polymer were calculated to be 24.72 and 6.63 $\mu A\,mM^{-1}\,cm^{-2}$, respectively. The limit of detection (LOD) for this MIP was found to be 0.3 μM at a signal to noise ratio (S/N) of 3. This MIP electrode was used as a biosensor for the detection of UA in the presence of ascorbic acid (AA) in a sample containing these species in the same concentrations as those in a human serum. The selectivity of MIP electrode is higher than that of NMIP electrode, and the values are 28.76 and 8.85, respectively. The results are substantiated by using cyclic voltammetry (CV), linear sweep voltammetry, amperometry, and scanning electron microscopy.

1. Introduction

There has been a very active research in this decade on molecular imprinting owing to its importance in a broad range of applications [1–3]. Molecularly imprinted polymer (MIP) is usually obtained by imprinting the polymer on a substrate through a template molecule. This template molecule is the analyte, which produces cavities specific to its detection in a bulk solution. After the extraction of the template molecule, the polymer matrix becomes complementary to the molecule and can rebind it with very high affinity and specificity.

During the last decade, molecularly imprinting technique has been developed as an analytical tool for the estimation of various electroactive species [4,5]. Efforts have been made to use MIPs for the detection of biomolecules [4–9]. MIP sensors can be substituted for enzyme-based sensors, because MIPs are enzyme mimics and can play the role of enzymes for catalyzing the reactions. In addition, they can be made at low cost and have higher thermal and chemical stability than that of enzymes [10,11]. There have been a number of reports on sensors based on molecularly imprinted conducting polymers, e.g., polyphenol [12], polypyrrole [13,14] and poly(phenylenediamines) [15]; in these reports, electrochemical methods, such as those with amperometry, impedance spectroscopy, and electrical capacitance were adopted. Malitesta et al. [16] reported the first successful application of electropolymerization for the preparation of MIPs. Ulyanova et al. [17] employed potentiodynamic electropolymerization technique to form a polyazine conducting polymer which was molecularly imprinted for the detection of theophyline. Benzimidazole compounds were determined in water samples by using MIPs [18] and performing the measurements in an HPLC system. Suedee et al. developed an MIP conductometric sensor for on-line sensing of haloacetic acids [19].

Uric acid (UA) is the principle final product of urine metabolism in human body [20] and is related to the occurrence of many disorders such as gout, hyperuricemia and Lesch–Nyhan syndrome [21]. Besides, UA is also one of the most important kidney calculus indices in human plasma. Thus, monitoring of the concentration of UA in human blood and urine is important for the prevention of the mentioned and other similar diseases. As mentioned above, electrochemical methods are very often used both at the stages of preparing and using the MIPs to detect the analytes. The common and well-developed electrochemical method for the detection of UA is based on the enzymatic approach. Uricase enzyme is

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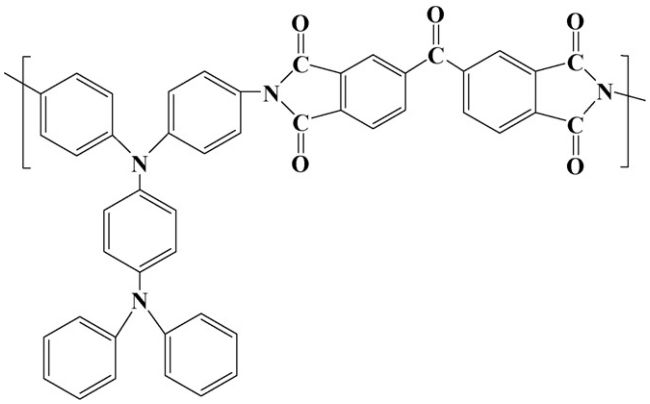
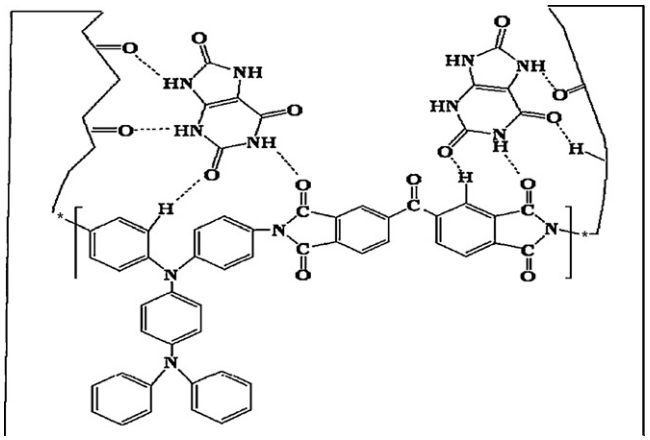


Fig. 1. Structure of poly(PD–BCD).

allowed to react with UA and the corresponding hydrogen peroxide is detected electrochemically. However, this method inherits some problems, such as high cost and low stability of enzymes; in addition the detection is indirect (detection of the reaction products). Several studies based on non-enzymatic methods have also been made to detect UA. These include the usage of carbon paste or activated glassy carbon electrodes [22,23], modified electrodes containing multi-wall carbon nanotubes (MWCNTs) [24] or a redox mediator [25].

The present study reports an electrochemical sensor for UA which is based on a novel molecularly imprinted amine–imide type conducting polymer. This is the first report using the polymer, denoted as poly(PD–BCD), as the MIP for sensing UA, in which the polymer was synthesized with N,N-bis(4-aminophenyl)-N′,N′-diphenyl-1,4-phenylenediamine (PD) and 3,3′,4,4′-benzophenonetetra carboxylic dianhydride (BCD). To the best of our knowledge, this is also the first quantitative study with regard to the content of any polymer to be used for optimizing the sensor for UA determination. Fig. 1 shows the structure of this polymer. Unlike the common approach of using a functional monomer and cross linker for the preparation of MIP, we directly applied for its preparation a sample of the polymer synthesized by us previously [26]. Incorporation of the analyte (UA) is purely a van der Waals interaction between the analyte and the sensor layer (polymer) (Scheme 1). The scheme shows formation of possible hydrogen bonds between the poly(PD–BCD) and the UA. Amine groups of UA can form hydrogen bonds with the ketone groups of poly(PD–BCD). In the same way, ketone groups of UA can also form hydrogen bonds with hydrogen atoms of benzene rings of the polymer. Such constitution can provide very strong adsorption force between UA and poly(PD–BCD). We studied the effects of varying the concentration of the novel polymer on its performance for the detection of UA. The optimum concentration of this polymer was evaluated in terms of the sensitivity of the MIP sensor for the detection of UA and in terms of the imprinting efficiency. This MIP electrode was also tested for the estimation of UA in the presence of ascorbic acid (AA) in a sample containing these species in the same concentrations as those of a human serum.



Scheme 1. Schematic representation of the formation of possible hydrogen bonds for the incorporation of UA in poly(PD–BCD).

2. Experimental

2.1. Chemicals and apparatus

The conducting polymer used for molecular imprinting was synthesized from N,N-bis(4-aminophenyl)-N′,N′-diphenyl-1,4-phenylenediamine and 3,3′,4,4′-benzo-phenone-tetracarboxylic dianhydride, and the synthesis steps were reported previously [26]. Uric acid (98%), ascorbic acid (99%), phosphate buffer saline tablet (PBS) and potassium chloride (KCl) were purchased from Aldrich (USA). 1-Methyl-2-pyrrolidinone (NMP, 99%) was purchased from Sigma (USA). Deionized water (>18 MΩ) was produced by Purelab Maximum (ELGA, UK). Indium–tin oxide (ITO, 10 Ω/□) glass was supplied by RiTdisplay Corporation (Hsinchu Industrial Park, Taiwan). All chemicals were used as received.

Amperometric measurements were carried out using potentiostat/galvanostat model CHI 440 (CH Instruments) and its compatible software. All electrochemical experiments were carried out at room temperature with a three-electrode system, containing a 50 mL glass cell with Ag/AgCl/saturated KCl reference electrode, platinum plate counter electrode and MIP or non-molecularly imprinted polymer (NMIP) working electrode.

2.2. Preparation of MIP and NMIP electrodes

The ITO glass was ultrasonically cleaned in a 0.1 M HCl for 5 min before using. The substrate was rinsed with deionized water and dried in air. After cleaning, epoxy tapes were fixed at the edges of the ITO-surface to restrict the active surface area to be 1.0 cm × 1.0 cm. For preparing the MIP electrodes, poly(PD–BCD) was mixed at various concentrations from 0.3 to 0.9 wt% with 1.5 mM UA dissolved in 1-methyl-2-pyrrolidinone, NMP, and the thus obtained solutions were drop-coated onto the ITO glass electrodes. The ITO electrodes were put into a vacuum oven and were subjected to a programmed temperature variation to remove the solvent from the electrodes. Then the temperature was kept at 60 °C for 4 h and raised to 80 °C for 2 h; finally the temperature was increased to 180 °C for 2 h. Such programmed temperature during the solvent removal is necessary to obtain uniform MIP films, and thereby higher detection currents in the amperometric analysis. After removing the solvent, Cu tape (3 M Company) was pasted at one edge of the conductive surface of the electrodes as a bus bar. Each electrode was then washed with deionized water to extract the UA template out of the polymer matrix, and then dried under nitrogen gas blow. The extracted MIP films were subjected to CV experiments within a potential window of 0.1–0.9 V (vs. Ag/AgCl/saturated KCl) for confirming the removal of the template. Disappearance of oxidation peak of the UA indicates its removal from the polymer. NMIP electrodes were also prepared in the same way, except that there was no addition of UA during their preparation.

2.3. Electrochemical measurements

Cyclic voltammetry (CV) was used to ascertain the complete removal of the template from the polymer film after the extrac-

tion with deionized water; the potential of the polymer electrode was swept from 0.1 to 0.9 V at a scan rate of 0.1 V s^{-1}. Furthermore, the charge capacities of MIP and NMIP electrodes were checked by comparing the areas under the pertinent CVs.

Linear sweep voltammetry (LSV) was performed to obtain the detecting potential of UA. The detection potential was determined by sweeping the potential of the MIP electrode (0.6 wt% of the polymer) in a window of potentials between 0.5 and 1.0 V. The corresponding background current was recorded in a 0.02 M phosphate buffer saline (PBS, pH 7.4), containing 0.1 M KCl as the supporting electrolyte, and the total current was recorded in a solution containing 1.5 mM UA. The net current was obtained by subtracting the background current from the total current. A proper operating potential was determined from the plateau part of the net current of the LSV curve. This way of estimating the operating potential was based on the fact that the plateau part corresponds to the diffusion control region of UA oxidation reaction and the sensing potential set at this region can obtain a current proportional to the concentration of UA.

UA was detected by the MIP and NMIP electrodes using an amperometric method. For this, steady-state current densities were obtained for MIP and NMIP electrodes at various concentrations of UA from 0 to 1.125 mM and the corresponding calibration curves were made with these values; the sensitivity and the detection limit were estimated from the calibration curve. Steady-state currents were also obtained by the amperometric method for the detection of 0.4 mM UA in the presence of 0.04 mM AA, which are their usual concentrations in a human serum; these experiments were simulated experiments intended to determine the selectivity of the MIP biosensor for UA in the presence of AA in a human serum. We mention here that the interference study of this work was restricted to AA and further study is envisaged with respect to other impurities such as dopamine and thiols.

We make here a simple comparison between amperometric and other electrochemical detection methods, to rationalize our preference of the former. Amperometric sensor has relatively short response time and can be designed easily as a portable sensor. Other special detection methods were used by several workers. Chou and Liu have used impedance detection method for sensing cholesterol with a thick film-MIP sensor. In this method a current decrease could be noticed when the analyte was attached to the MIP thick film [27]. A pulsed amperometric detection (PAD) method interprets the difference in currents obtained due to different bias potentials for dedoping and redoping as the analytical signal [28]; this method provides an accurate way for detecting the recombination of the target on the MIP electrode [29,30]. Potentiodynamic methods are usually suitable for sensing non-electroactive compounds, such as caffeine, protein and cholesterols. Both potentiodynamic and amperometric methods entail interferences, which disturb the current signal. This problem can be solved by modifying the electrodes with special materials such as Nafion® and mediators [31].

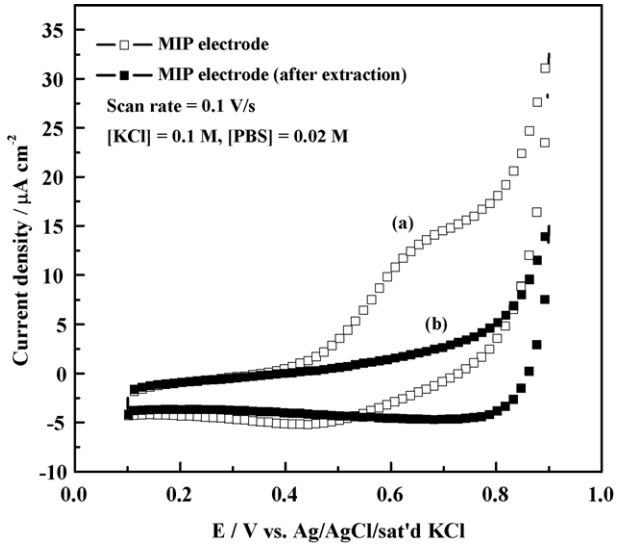


Fig. 2. CVs of the MIP electrode with 0.6 wt% of the polymer, in 0.1 M KCl + 0.02 M PBS (pH 7.4) at the scan rate of 0.1 V s^{-1}: (□) before extraction of UA and (■) after its extraction.

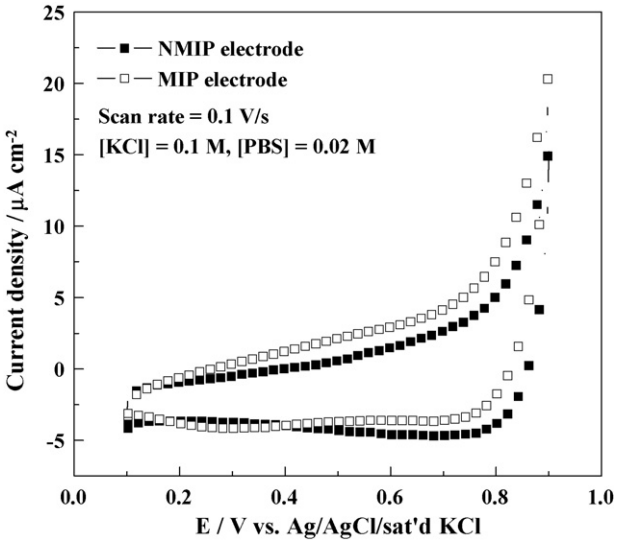


Fig. 3. Charge capacities of MIP electrode (□) and NMIP electrode (■) after the extraction of UA, both CVs being obtained in 0.1 M KCl + 0.02 M PBS (pH 7.4) at the scan rate of 0.1 V s^{-1}.

2.4. Morphological characterization

Scanning electron microscopy (SEM) images were obtained by Hitachi S-800 Field Emission SEM. For this purpose, the MIP and NMIP electrodes were sputtered with a layer of gold and the images were observed under a voltage of 20 kV.

3. Results and discussion

3.1. Performance evaluation of the MIP sensor

For evaluating the performance of a sensor, most important factors are sensitivity and selectivity. Fig. 2a shows the CV of the MIP electrode prepared with 0.6 wt% of the polymer in 0.1 M KCl and 0.02 M PBS. Fig. 2b shows the CV of the same electrode after the extraction of UA. The oxidation peak of UA can be clearly seen in Fig. 2a; after the extraction of UA its oxidation peak is absent in Fig. 2b, as desired and expected. This implies that UA molecules of the MIP electrode were removed after the extraction process. Further discussion will render evidence through SEM images that the extraction has removed only surface bound UA. In order to ensure that the same amount of poly(PD–BCD) remained after the extraction, the charge capacities of the MIP and NMIP electrodes were calculated using the CVs, as shown in Fig. 3. Calculation on the charge capacities was based on the integrated areas of the CVs. The charge capacities of the MIP and NMIP electrodes were found to be 4.812 and 4.475 μC, respectively, which clearly indicate that only UA was removed and not the polymer after the extraction process.

Before amperometric detection, the detection potential for UA was determined, using polarization curves of the MIP electrode in pure electrolyte and in electrolyte containing UA. Fig. 4 shows

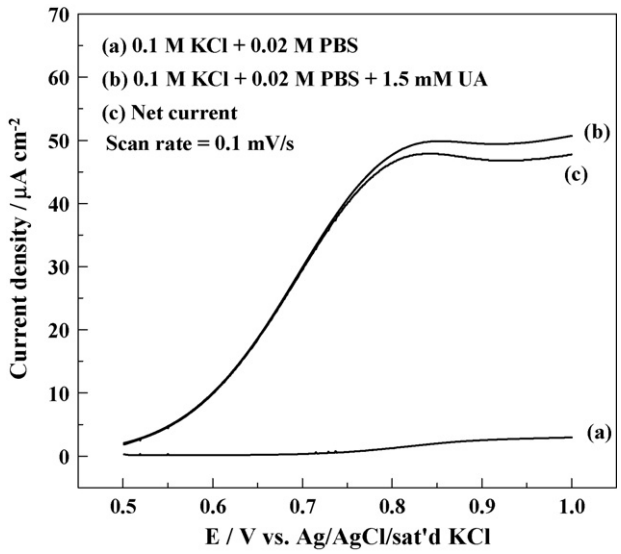


Fig. 4. Liner sweep voltammograms of MIP electrode in (a) 0.1 M KCl + 0.02 M PBS, and (b) 0.1 M KCl + 0.02 M PBS + 1.5 mM UA, at the scan rate of 0.1 V s^{-1}. This figure also shows the net current (c).

the LSVs of the electrode with 0.6 wt% of the polymer at a sweep rate of 0.1 mV s^{-1}. A plateau region between 0.8 and 1.0 V is the limiting current zone. Within this limiting current zone the current density is proportional to the concentration of an electroactive species. From these LSVs, the sensing potential was set at 0.85 V for obtaining amperometric steady-state current responses of UA. The mechanism is electro-oxidation of UA on the imprinted site of MIP modified electrode. At the limiting current region, diffusion of UA would control the whole system. The surface concentration of UA would be zero in this case and the current would depend on the bulk concentration of UA.

Amperometric experiments were carried out to examine the current responses of UA with the MIP and NMIP modified electrodes. The UA concentration was increased stepwise from 0 to 1.125 mM and the potential was fixed at 0.85 V. Fig. 5 shows the calibration curves of MIP and NMIP electrodes, both with a polymer content of 0.6 wt%. Both MIP and NMIP electrodes show good linear relationship between the current density and the concentration of UA for the entire concentration range of UA, with the correlation coefficients greater than 0.995 for both the electrodes. The calibration curves give the sensitivities of MIP and NMIP electrodes to be 24.72 and 6.63 μA mM^{-1} cm^{-2}, respectively. The current density responses of the MIP electrode are higher than those of the NMIP electrode at all the concentrations. The consistently enhanced current densities with MIP electrode reveal that imprinted sites were formed, most likely at the surface of the MIP electrode and they enabled the MIP electrode to recognize more UA molecules, than were recognized by the corresponding NMIP electrode. Indeed these sites or pores can be clearly seen in the corresponding SEM images in further discussions. When the template molecules were removed, a geometrically adopted polymer skeleton with fitting cavities and diffusion pathways for UA was left behind. The imprinting efficiency, defined as the ratio of the sensitivity of the MIP electrode to that of the NMIP electrode, is a property of an MIP electrode used for evaluating the recognition grade of the electrode. For the MIP electrode made by 0.6 wt% of the polymer, the imprinting efficiency is about 3.7, the highest in this study (Table 1). The limit of detection (LOD) for the MIP electrode was calculated to be 0.3 μM on the basis of signal to noise ratio (S/N) of 3. In real applications, the MIP electrode acts as the sensing electrode and the NMIP electrode as the reference one; this will be explained in further discussions.

3.2. Optimization of the polymer content for the MIP electrode

In our preliminary investigations we intended to study the effects of changing the polymer film thickness of the MIP electrode on the sensing performance of the electrode for UA. For this 0.3, 0.5, 0.6, 0.7, and 0.9 wt% of the polymer was mixed with 1.5 mM of UA for preparing the electrodes with different thicknesses of the polymer. Amperometric experiments were carried out with each of the thus fabricated electrodes, and the corresponding calibration curves were obtained. From these calibration curves sensitivities of the electrodes for UA detection were obtained. Sensitivities were also obtained for the corresponding NMIP electrodes. From the values of the sensitivities of the MIP electrodes and NMIP electrodes imprinting efficiencies of the MIP electrodes for various polymer concentrations were calculated. The performance parameters of the sensors with different polymer contents are listed in Table 1. Up to a polymer content of 0.6 wt%, the sensitivity of the MIP electrode shows an increase and reaches a maximum value of 24.72 μA mM^{-1} cm^{-2}. However, the sensitivity shows a decrease with higher polymer contents up to 0.9 wt%. This can be attributed to the inhibition to the electron conductivity in thick polymer films due to the presence of UA. It is known that electron conductivity cannot be sustained in a disproportionately thick conducting film

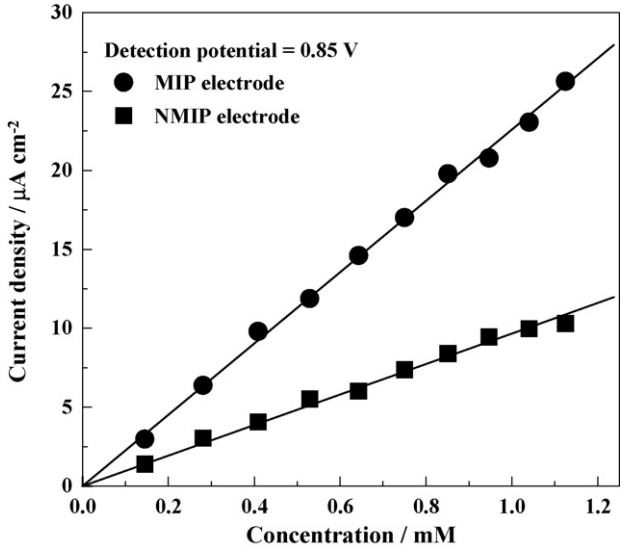


Fig. 5. Calibration curves of MIP (solid spheres) and NMIP (solid squares) electrodes with 0.6 wt% of the polymer in each case.

Table 1
Sensitivities of the MIP and NMIP electrodes and the corresponding imprinting efficiencies with different polymer contents of the electrodes.

Polymer content (wt%)	Sensitivity of MIP electrode (mA mM^{-1} cm^{-2})	Sensitivity of NMIP electrode (mA mM^{-1} cm^{-2})	Imprinting efficiency
0.3	22.59	9.67	2.34
0.5	22.94	6.53	3.51
0.6	24.72	6.63	3.73
0.7	17.43	7.16	2.43
0.9	0.03	8.54	0.0035

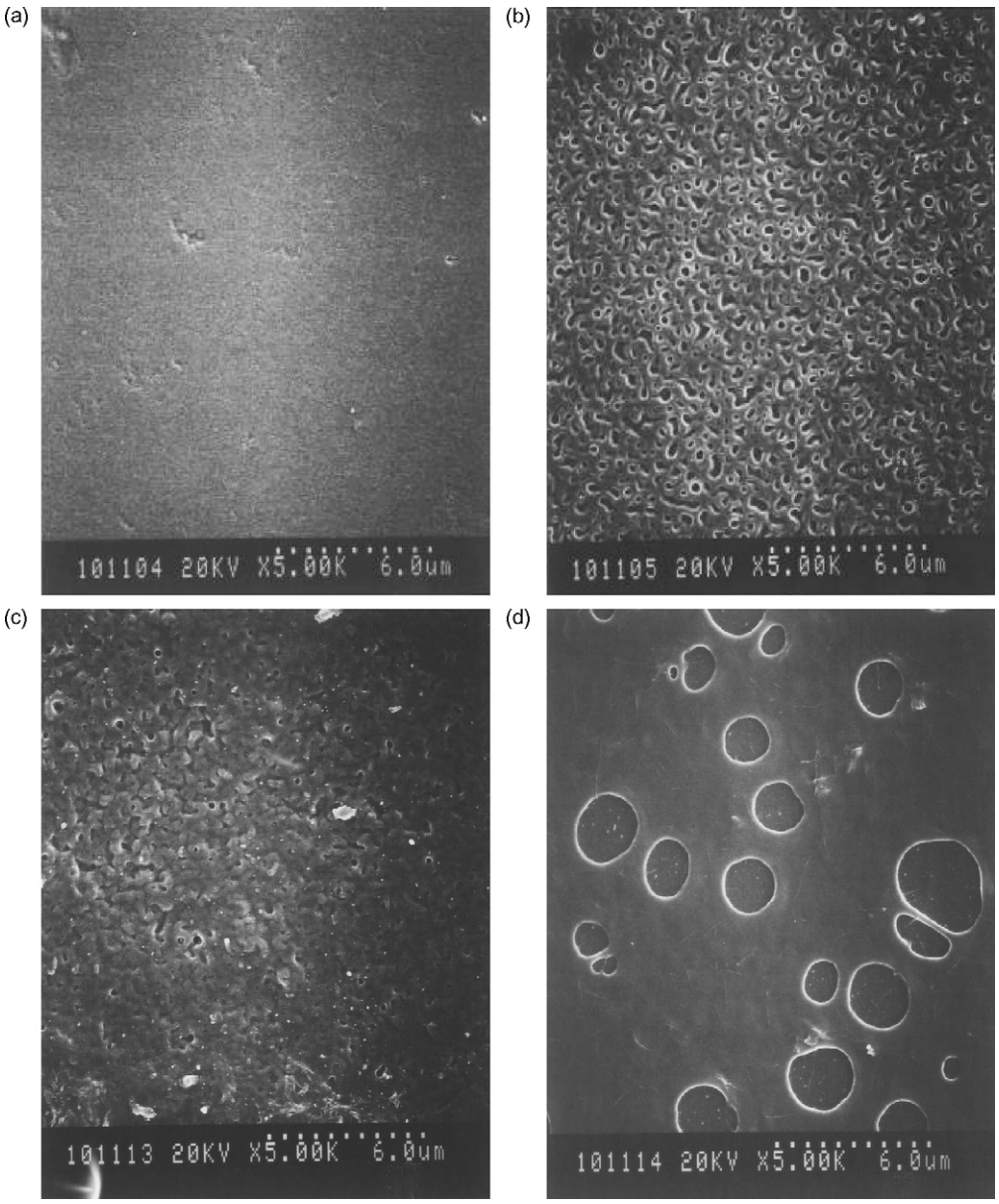


Fig. 6. SEM images of (a) NMIP electrode with 0.6 wt% of the polymer, (b) MIP electrode with 0.6 wt% of the polymer, (c) MIP electrode with 0.9 wt% of the polymer, and (d) MIP electrode with 0.3 wt% of the polymer.

on a substrate. In the case of high polymer contents, UA molecules are likely buried deep within the polymer film and the recognition sites are therefore much fewer on the surface of the polymer. As the imprint molecules are buried inside the polymer membrane, the effective electron diffusion coefficient within the membrane decreases, thereby decreasing the sensitivity of the pertinent electrode for UA detection. In order to verify the presence of these recognition sites, SEM was used to observe the surface morphol-

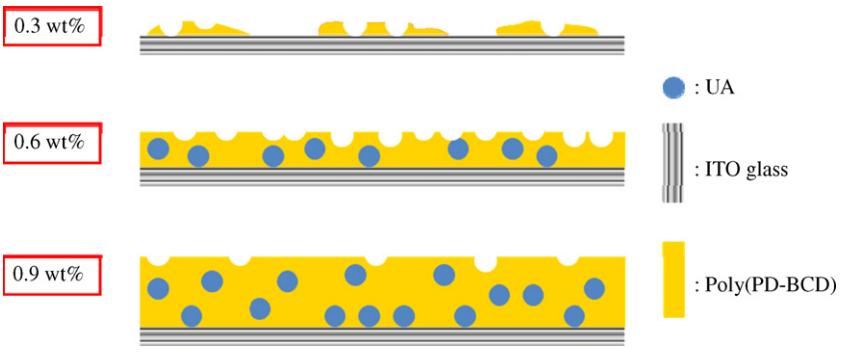


Fig. 7. Schematics representation of imprints of UA with different contents of the polymer.

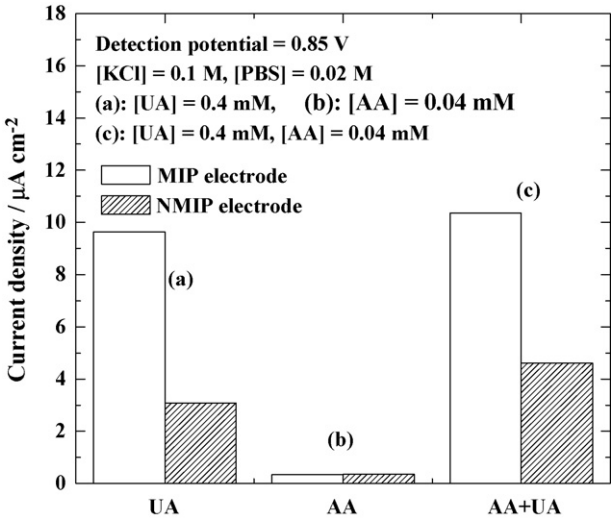


Fig. 8. Current density responses of the MIP and NMIP electrodes with 0.6 wt% of the polymer for (a) 0.4 mM of UA, (b) 0.04 mM of AA, and (c) 0.4 mM of UA + 0.04 mM of AA.

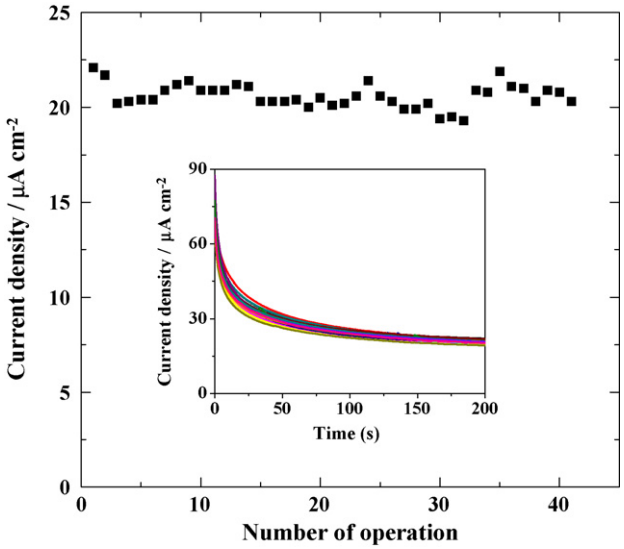


Fig. 9. Reusability of the MIP electrode with 0.6 wt% of the polymer for 40 operations.

ogy of MIP and NMIP electrodes. Fig. 6a and b is the SEM photos of NMIP and MIP electrodes, respectively, with 0.6 wt% of the polymer. The NMIP surface displays a relatively smooth surface. The MIP electrode shows disordered but mostly circular cavities with scattered large irregular pores, all obviously due to the templating process of the polymer with UA. These are the recognition sites of the MIP film for UA. When the polymer content is increased to 0.9 wt%, the MIP electrode shows insignificant number of pores and buried pores (Fig. 6c) with reference to those with 0.6 wt% of the polymer (Fig. 6b). Since the quantity of the template, i.e., UA used for each film is the same, the recognition sites created by the template should depend on the polymer content. Again, as the content of UA is the same in both the cases of MIPs with 0.6 and 0.9 wt% of the polymer, same extent of pores should have appeared in Fig. 6c (with 0.9 wt% of polymer) as that in Fig. 6b (with 0.6 wt% of polymer), if all the UA was bound to the surface of the electrode. As the surface pores are obviously not the same in both the figures, it implies that most of the UA molecules were buried in the thicker polymer film with 0.9 wt% of polymer and the subsequent recognition sites for UA in this case were much fewer than those of the film with 0.6 wt% of polymer. We believe that the MIP electrode with 0.9 wt% of polymer with buried UA has lost its usual polymer conductivity due to the damage of conductive paths for electron transfer, caused by the irregular burial of UA in the matrix of the polymer film. We attribute this non-conductivity behavior for the insignificant sensitivity of this MIP electrode for UA. With slightly higher content of the polymer, i.e., with 0.7 wt%, the MIP electrode showed lesser sensitivity of 17.43 $\mu A\,mM^{-1}\,cm^{-2}$, compared to that of the MIP electrode with 0.6 wt% of the polymer, for which the sensitivity was 24.72 $\mu A\,mM^{-1}\,cm^{-2}$. The damage caused for this MIP electrode with 0.7 wt% of the polymer was obviously less, as this content of the polymer was only slightly higher than that of the MIP electrode (0.6 wt%). Thus now it is clear from the results, that the optimum content of this polymer is 0.6 wt% for achieving the highest imprinting efficiency, and thereby sensitivity.

Table 1 also shows that the highest sensitivity of an NMIP electrode is with 0.3 wt% of the polymer. With 0.3 wt% of the polymer the electrode is virtually an ITO film because the polymer coverage is negligible. This is evidenced from SEM image in Fig. 6d. The figure provides solid evidence that the coverage of the ITO substrate with the polymer is little and polymer islands are indeed formed. The templating possibilities of UA with the films with 0.3, 0.6, and 0.9 wt% of the polymer are schematically represented in Fig. 7. The NMIP electrode with 0.5 wt% of the polymer is a "polymer modified" electrode and is different from the one with 0.3 wt% of polymer which has only islands of polymer, and otherwise is virtually an ITO bare electrode. Thus the trend of the sensitivity of NMIP electrode should be considered beginning from the NMIP electrode with 0.5 wt% of polymer. Increase in the polymer content has rendered a consistent increase in the sensitivity of the NIMP sensor, which can be attributed to the intrinsic nature of the conducting polymer. The optimum thickness of the polymer film for the NMIP electrode has apparently not reached and we have not used higher contents of polymer to investigate this, as the focus of our study is with MIP, for which the optimum thickness of this particular film is with 0.6 wt% of the polymer, as judged from its imprinting efficiency and sensitivity for UA. In absence of UA, the NMIP electrode with 0.9 wt% of polymer was apparently not damaged and the thickness can be probably increased for this type of NMIP electrode.

3.3. Selectivity of the MIP electrode for UA in the presence of AA

Intending to verify the sensitivity of this MIP sensor for the bioanalysis of human serum, a preliminary amperometric analysis was made for its specificity for UA in the presence of AA, which is the most important interference in electrochemical routine analysis of UA in a human serum. The normal concentration of AA in a human serum is about $45.8 \pm 16.2\ \mu M$ and is less influenced by dietary or smoke behavior [32]. In this study, the interference test was carried out with AA concentration set at 0.04 mM. Fig. 8 shows the individual and the coexistence current density responses of the MIP and NMIP electrode (0.6 wt% of polymer) for 0.4 mM of UA and 0.04 mM of AA. The figure indicates that the MIP electrode detects negligible current densities for AA. The current response of 0.04 mM AA is less than 7% of the total current obtained with 0.4 mM of UA and 0.04 mM of AA. The selectivity is defined as the sensitivity of UA divided by the sensitivity of AA using the same electrode. Higher selectivity implies better performance in sensing UA against AA. The selectivity of MIP electrode is about 28.76, this result shows that AA has low interference on the sensing of UA by the MIP electrode. Fig. 8 also shows the current density responses of the corresponding NMIP electrode for UA, AA and UA + AA. From this result, the selectivity of NMIP electrode is about 8.85. Compared to the case of

the MIP electrode, the current density response of the NMIP electrode is far lesser for UA, though it is the same for AA. Measured with the NMIP electrode, the current response for 0.4 mM of UA and 0.04 mM of AA is about 48% higher than that for 0.4 mM of UA. These results clearly demonstrate that the interference due to AA is higher in the case of the NMIP electrode with reference to the MIP electrode and thus the selectivity for UA with MIP electrode is far better than that with NMIP electrode. As mentioned already, in real applications, the MIP electrode can act as the sensing electrode and the NMIP electrode as the reference electrode. This may be rationalized as follows. Since the MIP electrode offers specific recognition sites for UA detection, the difference in sensitivities for the optimized MIP electrode (with 0.6 wt% of polymer) and its corresponding NMIP electrode is essentially to be attributed to the UA. Contribution to the sensitivity from the polymer film is eliminated in this way. Therefore the difference in sensitivities between the MIP electrode and its corresponding NMIP electrode is a better measure for the estimation of UA. The 7% interference due to AA in the estimation of UA in human serum would thus be further reduced. The reusability of the MIP electrode is shown in Fig. 9. The figure shows the steady-state current densities of the MIP electrode obtained for 40 different operations. The values were extracted from the steady-state currents of the MIP electrode versus time (shown as inset). The reusability was tested in a solution containing 0.02 M PBS, 0.1 M KCl and 1 mM of UA. After forty operations, an average current density was calculated to be $20.6 \pm 0.6\ \mu A\ cm^{-2}$. The error range of these results indicates that the MIP electrode is reusable for at least 40 operations, without a significant reduction in its sensitivity.

4. Conclusions

The novel polymer, denoted as poly(PD–BCD) was used to prepare a molecularly imprinted polymer (MIP) electrode using the template UA for its sensing. This MIP electrode was directly made from the polymer without a functional monomer and cross linker. As expected the MIP electrode showed about 3-fold higher sensitivity for UA than its counterpart, i.e., a non-molecularly imprinted (NMIP) electrode. The limit of detection (LOD) for this MIP sensor was found to be 0.3 μM at a signal to noise ratio (S/N) of 3. It is well established that the sensitivity of the MIP sensor depends on the thickness of the polymer, and the optimum thickness of the polymer is with 0.6 wt% of it, for obtaining the best sensitivity, and thereby imprinting efficiency. SEM photographs show clearly that the polymer at a concentration of more than 7 wt% had buried the UA in it, causing an adverse effect with respect to imprinting efficiency and thereby sensitivity of the pertinent MIP sensor. The porous structure caused by the UA in the polymer determines the sensitivity of the sensor. The MIP electrode can be used for a human serum as the biosensor with high selectivity for the estimation of UA in the presence of AA as the major impurity. The MIP-sensor was observed to be highly stable for at least forty times of operation.

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References

[1] W. Li, S.J. Li, Adv. Polym. Sci. 206 (2007) 191.
[2] X. Hu, Q. An, G. Li, S. Tao, J. Liu, Angew. Chem. Int. Ed. 45 (2006) 8145.
[3] H.J. Wang, W.H. Zhou, X.F. Yin, Z.X. Zhuang, H.H. Yang, X.R. Wang, J. Am. Chem. Soc. 128 (2006) 15954.
[4] K. Haupt, K. Mosbach, Chem. Rev. 100 (2000) 2495.
[5] P.K. Owens, L. Karlsson, E.S.M. Lutz, L.I. Andersson, Trac-Trends Anal. Chem. 18 (1999) 146.
[6] T.R. Ling, Y.Z. Syu, Y.C. Tasi, T.C. Chou, C.C. Liu, Biosens. Bioelectron. 21 (2005) 901.
[7] Y. Lu, C.L. Yan, S.Y. Gao, Appl. Surf. Sci. 255 (2009) 6061.
[8] W.X. Su, J. Rick, T.C. Chou, Microchem. J. 92 (2009) 123.
[9] A. Bossi, S.A. Piletsky, E.V. Piletska, P.G. Righetti, A.P.F. Turner, Anal. Chem. 73 (2001) 5281.
[10] G. Wulff, Chem. Rev. 102 (2002) 1.
[11] G. Wulff, T. Gross, R. Schonfeld, Angew. Chem. Int. Ed. 36 (1997) 1962.
[12] K.C. Ho, W.M. Yeh, T.S. Tung, J.Y. Liao, Anal. Chim. Acta 542 (2005) 90.
[13] A. Namvar, Biosens. Bioelectron. 22 (2007) 2018.
[14] A. Ramanavisius, A. Ramanaviciene, Electrochim. Acta 51 (2006) 6025.
[15] T.L. Panasyuk, V.M. Mirsky, S.A. Piletsky, O.S. Wolfbeis, Anal. Chem. 71 (1999) 4609.
[16] C. Malitesta, I. Losito, P.G. Zambonin, Anal. Chem. 71 (1999) 1366.
[17] Y.V. Ulyanova, A.E. Blackwell, S.D. Minteer, Analyst 131 (2006) 257.
[18] C. Cacho, E. Turiel, C. Pérez-Conde, Talanta 78 (2009) 1029.
[19] R. Suedee, W. Intakong, F.L. Dickert, Anal. Chim. Acta 569 (2006) 66.
[20] V.V.S. Eswara Dutt, H.A. Mottola, Anal. Chem. 46 (1974) 1777.
[21] H. Yamanaka, R. Togashi, M. Hakoda, C. Terai, S. Kashiwazaki, T. Dan, N. Kamatani, Adv. Exp. Med. Biol. 431 (1998) 13.
[22] X.H. Cai, K. Kalcher, C. Neuhold, B. Ogorevc, Talanta 41 (1994) 407.
[23] K. Shi, K.K. Shiu, Electroanalysis 13 (2001) 1319.
[24] Y.Y. Sun, J.J. Fei, K.B. Wu, S.S. Hu, Anal. Bioanal. Chem. 375 (2003) 544.
[25] S.B. Khoo, F. Chen, Anal. Chem. 74 (2002) 5734.
[26] S.H. Cheng, S.H. Hsaio, T.H. Su, G.S. Liou, Macromolecules 38 (2005) 307.
[27] L.C.S. Chou, C.C. Liu, Sens. Actuator B: Chem. 110 (2005) 204.
[28] A. Ramanaviciene, A. Ramanavisius, Anal. Bioanal. Chem. 379 (2004) 287.
[29] A. Ramanaviciene, A. Ramanavisius, Biosens. Bioelectron. 20 (2004) 1076.
[30] A. Ramanaviciene, A. Finkelsteinas, A. Ramanavisius, J. Chem. Educ. 83 (2006) 1212.
[31] P.Y. Chen, R. Vittal, P.C. Nien, K.C. Ho, Biosens. Bioelectron. 24 (2009) 3504.
[32] J. Lykkesfeldt, M. Viscovich, H.E. Poulsen, Free Radic. Biol. Med. 35 (2003) 1439.